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**CD133-enriched Xeno-Free human embryonic-derived neural stem cells expand rapidly in culture and do not form teratomas in immunodeficient mice.**

**Journal:** Stem Cell Res

**Publication Year:** 2014

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**PubMed link:** 25082219

**Funding Grants:** The Immunological Niche: Effect of immunosuppressant drugs on stem cell proliferation, gene expression, and differentiation in a model of spinal cord injury., Neural restricted, FAC-sorted, human neural stem cells to treat traumatic brain injury

**Public Summary:**

Common methods for the generation of human embryonic-derived neural stem cells (hNSCs) result in cells with potentially compromised safety profiles due to maintenance of cells in conditions containing non-human proteins (e.g. in bovine serum or on mouse fibroblast feeders). Additionally, sufficient expansion of resulting hNSCs for scaling out or up in a clinically relevant time frame has proven to be difficult. Here, we report a strategy that produces hNSCs in completely "Xeno-Free" culture conditions. Furthermore, we have enriched the hNSCs for the cell surface marker CD133 via magnetic sorting, which has led to an increase in the expansion rate and neuronal fate specification of the hNSCs in vitro. Critically, we have also confirmed neural lineage specificity upon sorted hNSC transplantation into the immunodeficient NOD-scid mouse brain. The future use or adaptation of these protocols has the potential to better facilitate the advancement of pre-clinical strategies from the bench to the bedside.

**Scientific Abstract:**

Common methods for the generation of human embryonic-derived neural stem cells (hNSCs) result in cells with potentially compromised safety profiles due to maintenance of cells in conditions containing non-human proteins (e.g. in bovine serum or on mouse fibroblast feeders). Additionally, sufficient expansion of resulting hNSCs for scaling out or up in a clinically relevant time frame has proven to be difficult. Here, we report a strategy that produces hNSCs in completely "Xeno-Free" culture conditions. Furthermore, we have enriched the hNSCs for the cell surface marker CD133 via magnetic sorting, which has led to an increase in the expansion rate and neuronal fate specification of the hNSCs in vitro. Critically, we have also confirmed neural lineage specificity upon sorted hNSC transplantation into the immunodeficient NOD-scid mouse brain. The future use or adaptation of these protocols has the potential to better facilitate the advancement of pre-clinical strategies from the bench to the bedside.

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